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13. ABSTRACT ***(Maximum 200 Words)***

This project is directed towards the design and synthesis of new drugs to treat breast cancer. Several naturally occurring substances have recently been discovered that have the same biological activity as the very important anticancer drug Taxol. We are using both computational and synthetic approaches to determine the parts of these very different compounds that are important for their biological activity. The determination of these "critical parts" could lead to the development of simpler structures that could be very powerful anticancer drugs. We have begun these studies by making new compounds based on a very promising taxol-like substance called epothilone. While the structures of the new compounds that we have prepared are very much like epothilone itself, we have not yet been able to prepare a simple structure with the same anticancer properties as taxol and epothilone. We are continuing our efforts to develop new anticancer drugs using this strategy.

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FOREWORD

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11-22-00

SIGNATURE P.I.

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INTRODUCTION

This proposal is directed towards the development of new chemotherapeutic agents based on the mechanism of action of Taxol™. The recent discovery of two other natural products, epothilone and discodermolide, that operate by the same unique mechanism of action as Taxol™, i.e., microtubule stabilization, provides a unique opportunity for a collaborative approach using synthetic and computational studies for the elucidation of the pharmacophore common to these structurally dissimilar substances. Such an advance could lead to the development of a novel family of breast cancer chemotherapeutics.

BODY

Significant progress has been achieved in realizing the first three tasks in the approved Statement of Work.

Task 1. The synthesis of both left- and right-hand halves **3** of epothilone **1** has been achieved (previous report). Since the last report, we have prepared new "right-hand" analogs **4-6** that differ from the previously described epothilone analog **3** in two important respects: 1) the presence of the conformationally restricting alkene in the ten-membered rings of **4** and **5**; and 2) the presence of side-chains in **5** and **6** that are intended to more closely mimic the hydrophobicity of the natural product epothilone **1** (Scheme 1; see Appendix).

The synthesis of the novel analogs **4-6** is outlined in Scheme 2. First, selective deprotection of the primary TBS ether in the presence of the two secondary TBS ethers in **7** was accomplished using PPTS in methylene chloride and methanol to give alcohol **8**. Substrate **8** was then subjected to hydrogenation conditions; treatment of **8** with four atmospheres of hydrogen and platinum oxide for 36 hours was required for the hydrogenation of this olefin. This provided cyclodecane **9** in quantitative yield. The primary alcohol was then oxidized in one step using Jones reagent to provide acid **10** in 62% yield. This acid was then coupled to the known alcohol **11** using DCC and DMAP in methylene chloride to provide ester **12** in 77% yield. This di-TBS ether was fully deprotected using TFA in methylene chloride to provide the desired ten-membered ring eastern hemisphere analog **3**. We also prepared analog **6** from acid **10** by first coupling to known alcohol **13** followed by silyl ether deprotection. This analog was prepared for the purpose of more closely imitating the natural product. Analog **6** contained three more carbons than analog **3**, giving it the same molecular formula as desoxyepothilone A. Analog **6** also possessed the natural stereochemistry at C15. We speculated, then, that since **6** should closely mimic epothilone in terms of its hydrophobicity, solubility could be ruled out as a factor for concern in the biological evaluation of these analogs.

Since the olefin metathesis reaction had cleanly produced only one olefin isomer, we were afforded simple means of preparing two additional eastern hemisphere analogs, as in Scheme 2. When tri-TBS ether **7** was subjected directly to Jones reagent, the primary TBS ether was deprotected and the resulting primary alcohol subsequently oxidized to provide β,γ-unsaturated acid **18** in good overall yield. It should be noted that alternative means of oxidizing the primary alcohol produced mixtures of **18** with its α,β-unsaturated isomer. Acid **18** could then be coupled to alcohol **11** and alcohol **13** to provide analogs **5** and **6**, respectively, after deprotection of the TBS ethers.

We have effected intensive study of the differences between these new compounds and the natural product **1** by both X-ray crystallographic analysis and NMR spectroscopy.

Task 2. In our developmental efforts thus far, we explored the use of an interface between hydrophobic and hydrophilic regions of a molecular dynamics simulation system as an deformable "receptor" for molecules of interest. This receptor was designed to respond to the presence of a test drug by adopting a shape that was complementary and energetically favorable for binding of the drug. By presenting the same shape simultaneously to two or more drugs, we effectively searched the conformational space of this interface for a conformation that was most complementary to both drugs. The conformation of the drugs that simultaneously bound to this common interface would, in turn, constitute the hypothetical active or bound conformation of the drug. The principal determinants of the drug that mediated its contact with the interface would constitute the hypothetical active pharmacophore of the two drugs.

A fully functional version of program to implement this strategy was developed, but it proved to be too computationally intensive for practical use. Projections based on our initial calculations indicated that an impractical amount of computer power would be required to even run test cases. For this reason, we have now changed our strategy to eliminate the need for large numbers of hydrophobic and hydrophilic particles. In our new approach, the fast "RigFit" algorithm (1) is used to evaluate the similarity of two or more molecules that are independently exploring their own conformational space by means of molecular dynamics simulation. The RigFit similarity function substitutes for the deformable interface of our original approach, making it necessary to search only the conformational space of the test compounds. Conservatively, this should accelerate the search for a common pharmacophore in two compounds by several orders of magnitude because the interface comprised over 80% of the particles in our first system, and the size of the computational task roughly scales with the square of the number of particles.

Task 3. Analogs **3**, **4**, **5** and **6** were sent to Dr. Susan Horwitz at the Department of Molecular Pharmacology at the Albert Einstein College of Medicine for biological testing. None of these compounds displayed activity in tubulin turbidity measurement experiments or in tubulin depolymerization experiments.

Relevance to the Original Hypothesis: The lack of biological activity in the new analogs that we have prepared indicates that the partial epothilone structures designed to date do not contain enough of the functionality of the natural product for biological activity. These new compounds rule out the role of hydrophobicity *per se* in the observed lack of biological activity. The remainder of our synthetic efforts will therefore be devoted to the construction of the conformationally restricted macrocycle **2** (Scheme 1).

KEY RESEARCH ACCOMPLISHMENTS:

* New analogs of the potent antitumor substance epothilone have been prepared and the role of hydrophobicity has been elucidated

* A new approach to the establishment of a common pharmacophore for structurally dissimilar substances using RigFit is being evaluated

* Biological evaluation of these new compounds (cytotoxicity and tubulin polymerization) indicates that they are NOT biologically active.

REPORTABLE OUTCOMES:

A publication is now in preparation describing the molecular modeling approach to the design and synthesis of novel epothilone analogs;

This work was presented at the Warner Lambert Lectures at Michigan State University and at the 2000 Gordon Research Conference on Heterocyclic Chemistry;

Training has been provided on this project to Ms. Erin Mattingly, who has taken a position as a MS chemist at Merck and Co.

CONCLUSIONS

We have established that the originally proposed partial structures of epothilone are not sufficient for the biological activity of the natural product. Modification of these structures to enhance hydrophobicity has not led to increased biological activity, in spite of the congruence of these partial structures with epothilone as determined by X-ray crystallographic and NMR analysis. The final synthetic goal of this project is to prepare the more complex analog **2**, which more closely resembles the natural product.

The preparation of conformationally restricted analogs with biological activity would represent an important advance that could be used for the refinement of the requisite SAR for the pharmacophore model. As stated previously, the development of such a model would provide the basis for the development of a new family of breast cancer chemotherapeutic agents.

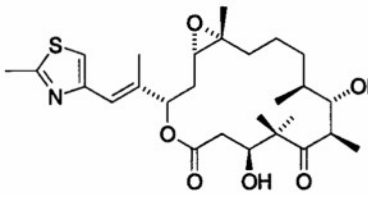
REFERENCES

C. Lemmen, C. Hiller, T. Lengauer, "RigFit: A New Approach to Superimposing Ligand Molecules." *J.Comp.Aid.Mol.Des.* 1998, **12**, 491-502.

APPENDICES

Schemes 1 and 2, as well as a CV for the PI.

Scheme 1



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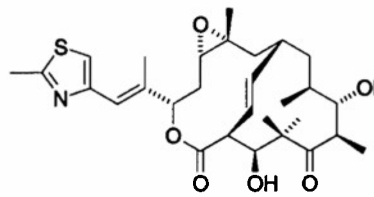
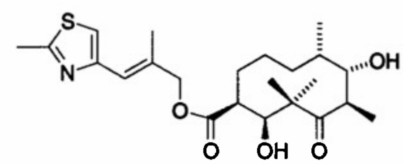
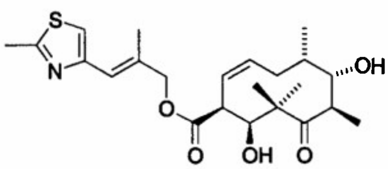


2:
Conformationally-
Constrained
Epothilone Analog



3: Right-Hand Partial Structure



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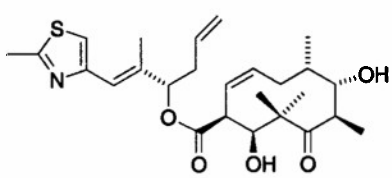

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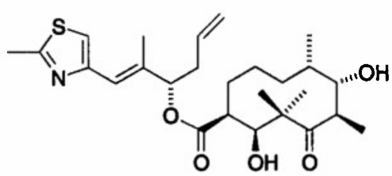
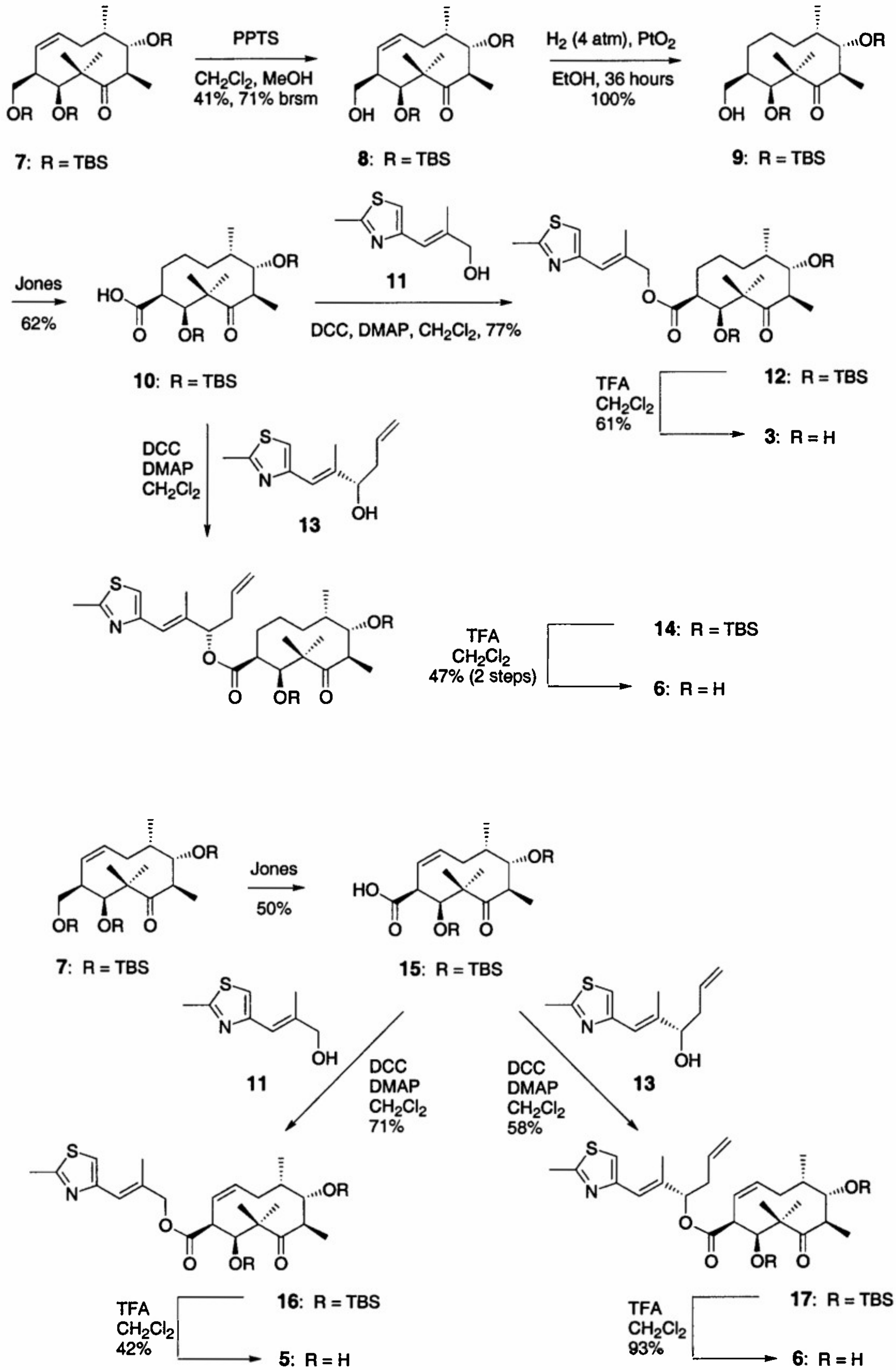


6

Scheme 2


PPTS
CH_2Cl_2, MeOH
41%, 71% brsm
H_2 (4 atm), PtO_2
EtOH, 36 hours
100%
7: R = TBS
8: R = TBS
9: R = TBS
Jones
62%
10: R = TBS
11
DCC, DMAP, CH_2Cl_2, 77%
12: R = TBS
TFA
CH_2Cl_2
61%
3: R = H
DCC
DMAP
CH_2Cl_2
13
TFA
CH_2Cl_2
47% (2 steps)
14: R = TBS
6: R = H
7: R = TBS
Jones
50%
15: R = TBS
11
DCC
DMAP
CH_2Cl_2
71%
13
DCC
DMAP
CH_2Cl_2
58%
16: R = TBS
TFA
CH_2Cl_2
42%
5: R = H
17: R = TBS
TFA
CH_2Cl_2
93%
6: R = H

CURRICULUM VITAE

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BIRTH DATE: [redacted]

EDUCATION:

Post-doctoral: Columbia University. January 1982-August 1983.
Research Director: Professor Ronald Breslow.

Graduate: Columbia University. September 1977-December 1981.
M.A. 1978, M.Phil., Ph.D. 1981.
Thesis Advisor: Professor Gilbert Stork.

Undergraduate: Harvard College. September 1973-June 1977.
A. B. cum laude in Chemistry, 1977.

PROFESSIONAL EXPERIENCE:

Professor, University of Pennsylvania
Department of Chemistry, July 1996-

Founding Member, University of Pennsylvania
Center for Cancer Pharmacology, May 1998-present

Visiting Professor, University of Paris-Sud, June 2001

Associate Professor, University of Pennsylvania,
Department of Chemistry, July 1990-June 1996
Member, University of Pennsylvania Cancer Center,
July 1993-present

Assistant Professor, University of Chicago,
Department of Chemistry, September 1983-June 1990

AWARDS & HONORS:

American Chemical Society Cope Scholar Award, 2000
Parke-Davis Lecturer, Michigan State University, 2000
Chairman, Philadelphia Organic Chemists' Club, 1995
H. Martin Friedmann Lecturer, Rutgers University, 1993
American Cyanamid Young Faculty Award, 1989-1992
NIH-NCI Research Career Development Award, 1988-1993
Alfred P. Sloan Research Fellow, 1987-1989
Merck Foundation Award for Faculty Development, 1985
American Cancer Society Postdoctoral Fellow, 1982-1983

RESEARCH SUPPORT

ACTIVE

CA 40250-08A2 (Winkler) 2/5/98-12/31/00 20%
National Institutes of Health $191,555 (direct costs/year)
Strategies for the Synthesis of Antitumor Compounds
This proposal is directed towards the development of new approaches to the construction of the naturally occurring substances manzamine and ingenol.

PC970475 (Winkler) 9/1/98-2/28/01 20%
DOD Prostate Cancer Research Program $114,960 (direct costs/year)
Design and Synthesis of New Prostate Cancer Chemotherapeutic Agents
This proposal is directed towards design and synthesis of new prostate cancer chemotherapeutic agents based on taxol and epothilone. The synthetic work in the DOD PC grant is directed towards the synthesis of the left- and right-hand halves of an X-ray based bridged bicylic analog of epothilone

BCRP-971965 (Winkler) 7/15/98-7/14/01 20%
DOD Breast Cancer Research Program (IDEA) $69,905 (direct costs/year)
Design and Synthesis of New Breast Cancer Chemotherapeutic Agents
This proposal is directed towards design and synthesis of new breast cancer chemotherapeutic agents based on taxol and epothilone. The synthetic work in this proposal is directed towards the synthesis of bicyclic analogs of epothilone

PA98-07-17 (Winkler) 7/01/00-6/30/01
Pennsylvania Department of Health $30,000 (direct costs)
Support for a graduate student who is to study the synthesis of microtubule stabilizing chemotherapeutics.

PROFESSIONAL ACTIVITIES

Consultant, Wyeth-Ayerst Pharmaceuticals (1998-)
Associate Editor, *Organic Letters* (1999-)

INVITED LECTURES SINCE 1990:

Merck, Sharp & Dohme (West Point, PA)
Smith, Kline and Beckmann
Invited Lecturer, Symposium on Organic Synthesis, Great Lakes Regional ACS Meeting, Dekalb, Illinois Invited Lecturer, Molecular Recognition Meeting, Office of Naval Research, Charleston, S.C
Invited Lecturer, Symposium on Heterocyclic Chemistry, National ACS Meeting, Washington, D.C
Squibb Institute for Medical Research (Princeton, NJ)
University of Rochester
Squibb Institute for Medical Research (New Brunswick, NJ)
Boehringer-Ingelheim Pharmaceuticals
Brandeis University
University of Delaware
ICI Pharmaceuticals
New York Academy of Sciences

North Jersey ACS Meeting
Invited Lecture, 1992 Meeting of the American Society for Photobiology
Organizer and Lecturer, Symposium on Studies Toward the Total Synthesis of Taxol, National ACS Meeting, San Francisco, CA. (April 8, 1992)
Dupont Agricultural Products
Burroughs Wellcome
University of Virginia
Sandoz Institute
Sterling Winthrop
Bryn Mawr College
Invited Lecturer, Symposium on Organic Chemistry, Great Lakes Regional ACS Meeting, Ann Arbor, Michigan
Invited Lecturer, Symposium on Organic Synthesis, Middle Atlantic Regional ACS Meeting, Baltimore, Maryland
Technion-Israel Institute of Technology
Pfizer Central Research
Sandoz Institute
Hebrew University of Jerusalem
R. W. Johnson
University of Montreal
Plenary Lecturer, Wyeth-Ayerst Fourth Annual Chemical Sciences Symposium
Merck (West Point, PA)
American Cyanamid
Rhone-Poulenc Agricultural
Plenary Lecture, Interamerican Photochemical Society
University of Maryland
R. W. Johnson Pharmaceutical Research
Wyeth-Ayerst
Sepracor
Boehringer-Ingelheim
Florida State University
Northwestern University
UCLA
University of Minnesota
Parke-Davis
Pfizer
Penn State University
Smith Kline Beecham
Temple University
Amgen
University of Chicago
Dupont Pharmaceuticals
Invited Speaker, Symposium on Solid Support Chemistry, Middle Atlantic Regional ACS Meeting, May 1999
Plenary Lecturer, Symposium on Heterocycles, Canadian Institute of Chemistry, June 1999
Invited Speaker, Gordon Conference on Heterocycles, July 2000
University of Western Ontario
Boehringer-Ingelheim, Montreal
Villanova University
Johnston Mathey
Lederle Laboratories
Genetics Institute
University of Pittsburgh
Merck-Frosst Lecturer, University of Sherbrooke

Parke Davis Lecturer, Michigan State University
Bristol-Myers Squibb Lecturer, MIT
Albany Molecular Sciences
University of California, Irvine

PUBLICATIONS :

1. L. Blaszczak, J. Winkler, S. O'Kuhn, "A New Synthesis of Olefins from Ketones via Coupling of Lithium Dialkylcuprates with Enol Phosphate Diesters," *Tetrahedron Lett.* **1976**, 4405-4408.

2. G. Stork, C. Shiner, J. Winkler, "Stereochemical Control of the Internal Michael Reaction. A New Construction of trans-Hydrindane Systems," *J. Am. Chem. Soc.* **1982**, *104*, 310-312.

3. G. Stork, J. Winkler, C. Shiner, "Stereochemical Control of Intramolecular Conjugate Addition. A Short, Highly Stereoselective Synthesis of Adrenosterone," *J. Am. Chem. Soc.* **1982**, *104*, 3767-3768.

4. G. Stork, J. Winkler, N. Saccomano, "Stereochemical Control in the Construction of Vicinally Substituted Cyclopentanes and Cyclohexanes. Intramolecular Conjugate Addition of β-Ketoester Anions," *Tetrahedron Lett.* **1983**, 465-468.

5. J. Winkler, E.Coutouli-Argyropoulou, R. Leppkes, R. Breslow, "An Artificial Transaminase Carrying A Synthetic Macrocyclic Binding Group," *J. Am. Chem. Soc.* **1983**, *105*, 7198-7199.

6. W. Weiner, J. Winkler, S. Zimmerman, R. Breslow, "Mimics of Tryptophan Synthetase and of Biochemical Dehydroalanine Formation," *J. Am. Chem. Soc.* **1985**, *107*, 4093-4094.

7. R. Breslow, A. W. Czarnik, M. Lauer, H. Leppkes, J. Winkler, S. Zimmerman, "Mimics of Transaminase Enzymes," *J. Am. Chem. Soc.* **1986**, *108*, 1969-1979.

8. J. Winkler, V. Sridar, "Stereochemical Control of Transannular Radical Cyclizations. A New Approach to the Synthesis of Linearly Fused Cyclopentanoids," *J. Am. Chem. Soc.* **1986**, *108*, 1708-1709.

9. J. Winkler, J. Hey, P. Williard, "Inside-Outside Stereoisomerism: A Synthesis of *trans*-Bicyclo[5.3.1]undecan-11-one," *J. Am. Chem. Soc.* **1986**, *108*, 6425-6427.

10. J. Winkler, P. Hershberger, J. Springer, "A Stereoselective Synthesis of the Azaspiroundecane Ring System of (-)-Histrionicotoxin from (+)-Glutamic Acid," *Tetrahedron Lett.* **1986**, 5177-5180.

11. J. Winkler, J. Hey, S. Darling, "Studies Directed Towards the Synthesis of the Taxane Diterpenes: A Remarkable Rearrangement," *Tetrahedron Lett.* **1986**, 5959-5962.

12. J. Winkler, J. Hey, F. Hannon, P. Williard, "Intramolecular Photoaddition of Dioxolenones. An Efficient Method for the Synthesis of Medium-Sized Rings," *Heterocycles* **1987**, *25*, 55-60.

13. J. Winkler, K. Deshayes, "Photodynamic Macrocycles," *J. Am. Chem. Soc.* **1987**, *109*, 2190-2191.

14. J. Winkler, K. Henegar, P. Williard, "Inside-Outside Stereoisomerism II. Synthesis of the Carbocyclic Ring System of the Ingenane Diterpenes *via* the Intramolecular Dioxolenone Photocycloaddition," *J. Am. Chem. Soc.* **1987**, *109*, 2850-2851.

15. K. Henegar, J. Winkler, "A New Method for the Synthesis of Dioxolenones via the Carboxylation of Ketone Enolates with Anisyl Cyanoformate," *Tetrahedron Lett.* **1987**, 1051-1054.

16. J. Winkler, C. Muller, R. Scott, "A New Method for the Formation of Nitrogen-Containing Ring Systems *via* the Intramolecular Photocycloaddition of Vinylogous Amides. A Synthesis of Mesembrine," *J. Am. Chem. Soc.* **1988**, *110*, 4831-4832.

17. J. Winkler, J. Hey, P. Williard, "Inside-Outside Stereoisomerism III. The Synthesis of *trans*-Bicyclo[4.3.1]Decan-10-one," *Tetrahedron Lett.* **1988**, 4691-4694.

18. J. Winkler, V. Sridar, "Eight-Membered Ring Templates for Stereoselective Radical Cyclizations," *Tetrahedron Lett.* **1988**, 6219-6222.

19. J. Winkler, K. Deshayes, B. Shao, "Photodynamic Systems for Metal Ion Transport," *J. Am. Chem. Soc.* **1989**, *111*, 769-770.

20. J. Winkler, P. Hershberger, "A Stereoselective Synthesis of (-)-Perhydrohistrionicotoxin," *J. Am. Chem. Soc.* **1989**, *111*, 4852-4856.

21. J. Winkler, V. Sridar, L. Rubo, J. Hey, N. Haddad, "Inside-Outside Stereoisomerism IV. An Unusual Rearrangement of the trans-Bicyclo[5.3.1]Undecan-11-yl Radical," *J. Org. Chem.* **1989**, *54*, 3004-3006.

22. J. Winkler, C. Lee, L. Rubo, C. Muller, P. J. Squattrito, "Stereoselective Synthesis of the Tricyclic Skeleton of the Taxane Diterpenes. The First C-Silylation of a Ketone Enolate," *J. Org. Chem.* **1989**, *54*, 4491-4493.

23. J. Winkler, V. Sridar, M. Siegel, "Ten-Membered Ring Templates for Stereoselective Radical Cyclizations," *Tetrahedron Lett.* **1989**, 4943-4946.

24. J. Winkler, C. Muller, J. Hey, R. Ogilvie, N. Haddad, P. Squattrito, P. Williard, "The Effect of Chromophore Transposition on the Stereochemical Outcome of the Intramolecular Dioxenone Photocycloaddition Reaction," *Tetrahedron Lett.* **1989**, 5211-5214.

25. J. Winkler, N. Haddad, R. Ogilvie, "Intramolecular Photocycloaddition and Retro-Mannich Fragmentation of Tertiary Vinylogous Amides," *Tetrahedron Lett.* **1989**, 5703-5704.

26. J. Winkler, M. Finck-Estes, "Carbon-Carbon Bond Formation Under Aqueous Reaction Conditions Using Sulfonium and Selenonium Salt Electrophiles," *Tetrahedron Lett.* **1989**, 7293-7296.

27. J. Winkler, R. Scott, P. Williard, "Asymmetric Induction in the Vinylogous Amide Photocycloaddition. A Formal Synthesis of Vindorosine," *J. Am. Chem. Soc.* **1990**, *112,* 8971-8975.

28. J. Winkler, B. Hong, "Inside-Outside Stereoisomerism V. Synthesis and Reactivity of Bicyclo[n.3.1]alkanones with trans Intrabridgehead Stereochemistry" *J. Am. Chem. Soc.* **1991**, *113,* 8839-8846.

29. J. Winkler, E. Gretler, "Stereoselective Cyclopropanation of Homoallylic Alcohols. Formal Attachment of a Cyclopropane to a Preexisting Ring," *Tetrahedron Lett.* **1991**, 5733-36.

30. J. Winkler and D. Subrahmanyam, "Studies Directed Towards the Synthesis of Taxol: Preparation of C-13 Oxygenated Taxane Congeners," *Tetrahedron* **1992**, *48*, 7049-7056.

31. J. Winkler, E. Gretler, P. Williard,"Studies Directed Towards the Synthesis of the Ingenane Diterpenes. An Unexpected Synthesis of trans-Bicyclo[5.3.0]Decanes," *J. Org. Chem.* **1993**, *58,* 1973-1975.

32. J. Winkler, B. Hong, A. Bahador, M. Kazanietz, P. Blumberg, "Synthesis of Ingenol Analogs with Affinity for Protein Kinase C," *Bioorg. Med. Chem. Lett.* **1993**, *3,* 577-580.

33. J. Winkler, B. Shao, "On the Stereoselectivity of the Intramolecular Dioxenone Photocycloaddition Reaction," *Tetrahedron Lett.* **1993**, 3355-3358.

34. J. Winkler, M. Siegel, and J. Stelmach, "A Highly Stereoselective Approach to the Synthesis of the Manzamine Alkaloids via the Intramolecular Vinylogous Amide Photocycloaddition," *Tetrahedron Lett.* **1993**, 6509-6512.

35. J. Winkler, K. Deshayes and Bin Shao, "Photochemical Binding, Release and Transport of Metal Ions." In *Bioorganic Photochemistry,* **1993**, *Volume II*, H. Morrison, Ed., Wiley, New York, Chapter 3, pp. 169-196.

36. J. Winkler, M. Siegel, "A Novel Photochemical Synthesis of Pyrroles from β-Ketoviny-logous Amides," *Tetrahedron Lett.* **1993**, 7697-7700.

37. M. Siegel and J. Winkler, "Photochemistry of Enamines and Enaminones" In *The Chemistry of Enamines,* **1994**, S. Patai and Z. Rappaport, Eds., Wiley, New York, 637-679.

38. J. Winkler, K. Henegar, B. Hong and P. Williard, "Inside-Outside Stereoisomerism. 6. Synthesis of *trans*-Bicyclo[4.4.1]Undecan-11-one and the First Stereoselective Construction of the Ingenane Nucleus," *J. Am. Chem. Soc.* **1994**, *116,* 4183-4188.

39. J. Winkler, B. Hong, "*Trans*-Bicyclo-[5.3.1]undecan-11-one," in Photochemical Key Steps in Organic Synthesis **1994**, J. Mattay and A. Griesbeck, Eds., VCH, Weinheim, 109-111.

40. K. Davis, T. Berrodin, T., J. Stelmach, J. Winkler, M. Lazar, "Endogenous RXRs can function as hormone receptors in pituitary cells," *Molecular and Cell Biology* **1994**, *14*, 7105-7110.

41. J. Winkler, S. Kim, K. Condroski, A. Asensio, K. N. Houk, "Stereoselective Synthesis of Polycyclic Ring Systems via the Tandem Diels-Alder Reaction," *J. Org. Chem.* **1994**, *59*, 6879-6881.

42. J. Winkler, B. Hong, "Transannular Radical Reactions in Bicycloalkanes with 'Inside-Outside' Stereochemistry. An Unusual Bridgehead Hydroxylation," *Tetrahedron Lett.* **1995**, 683-686.

43. J. Winkler, H. Kim, S. Kim, "A Highly Efficient Synthesis of Taxanes via the Tandem Diels-Alder Reaction," *Tetrahedron Lett.* **1995**, 687-691.

44. J. Winkler, B. Hong, A. Bahador, M. Kazanietz, P. Blumberg, "Methodology for the Synthesis of 3-Oxygenated Ingenanes--The First Ingenol Analogs with High Affinity for Protein Kinase C," *J. Org. Chem.* **1995**, *60*, 1381-1390.

45. J. Winkler, S. Bhattacharya, F. Liotta, R. Batey, G. Heffernan, D. Cladingboel, R. Kelly, "Stereoselective Synthesis of A Synthon for the A-Ring of Taxol from R-(+)-Verbenone," *Tetrahedron Lett.* **1995**, 2211-2215.

46. J. Winkler, B. Hong, S. Kim, N. Lewin, P. Blumberg, "On the Protein Kinase C Pharmacophore: Synthesis and Biological Activity of 4-Hydroxylated Analogs of Ingenol," *Synlett* **1995**, 533-535.

47. H. Li, S. Narasimhulu, L. Havran, J. Winkler, T. Poulos, "Crystal Structure of Cytochrome P-450 Complexed with Its Catalytic Product, 5-Exo-Hydroxycamphor," *J. Am. Chem. Soc.* **1995**, *117*, 6297-6299.

48. J. Winkler, C. Mazur, and F. Liotta, "[2+2]Photocycloaddition-Fragmentation Strategies for the Synthesis of Natural and Unnatural Products," *Chem. Rev.* **1995**, *95*, 2003-2020.

49. J. Winkler, "The Tandem Diels-Alder Reaction," *Chem. Rev.* **1996**, *96*, 167-176.

50. J. Winkler, J. Stelmach, J. Axten, "Two Highly Efficient Syntheses of Scalemic Azocines," *Tetrahedron Lett.* **1996**, 4317-4320.

51. J. Winkler, S. Bhattacharya, R. Batey, "Synthesis of a Taxinine Congener via the Intramolecular Diels-Alder Cycloaddition," *Tetrahedron Lett.* **1996**, 8069-8072.

52. J. Winkler, J. Holland, D. Peters, "Synthesis of Cyclopropyl Taxane Analogs via Sequential Diels-Alder Reactions," *J. Org. Chem.* **1996**, *61*, 9074-9075.

53. J. Winkler and P. Axelsen, "A Model for the Taxol/Epothilone Pharmacophore," *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2963-2966.

54. J. Winkler, H. Kim, S. Kim. K. Ando, K. Houk, "Stereoselective Synthesis of the Taxane Ring System via the Tandem Diels-Alder Cycloaddition," *J. Org. Chem.* **1997**, *62,* 2957-2962.

55. J. Winkler, J. Stelmach, M. Siegel, N. Haddad, J. Axten, W. Dailey, "An Approach to the Synthesis of the Manzamine Alkaloids via the Vinylogous Amide Photocycloaddition-retro-Mannich Fragmentation-Mannich Closure Cascade," *Isr. J. Chem.* **1997**, *37,* 47-67.

56. S. Kim, J. Winkler, "Approaches to the Synthesis of Ingenol," *Chem. Soc. Rev.* **1997**, *26,* 387-400.

57. J. Winkler, E. Doherty, "Control of Relative Stereochemistry of Quaternary Carbon Centers via the Intramolecular Dioxenone Photocycloaddition: An Approach to the Synthesis of Saudin," *Tetrahedron Lett.* **1998**, 2253-2256.

58. J. Winkler, C. Bowen, V. Michelet, "Photodynamic Fluorescent Metal Ion Sensors with ppb Sensitivity," *J. Am. Chem. Soc.* **1998**, *120*, 3237-3242.

59. S. Narasimhulu, L. Havran, P. Axelsen, J. Winkler, "Interactions of Substrate and Product with Cytochrome P450," *Arch. Biochem. Biophys.* **1998**, *353*, 228-238.

60. J. Winkler, J. Axten, H. Hammach, Y. Kwak, M. Lucero, K. Houk, "Stereoselective Synthesis of the Tetracyclic Core of the Manzamine Alkaloids via the Vinylogous Amide Photocycloaddition Cascade: A Remarkable Effect of Azocine Unsaturation on the Stereochemical Outcome of the Photocycloaddition Reaction," *Tetrahedron* **1998**, *54*, 7045-7056.

61. J. Winkler, W. McCoull, "Diels-Alder Reaction on Solid Supports Using Polymer-Bound Oxazolidinones," *Tetrahedron Lett.* **1998**, 4935-4936.

62. J. Winkler, J. Axten, "The First Total Syntheses of Ircinol A, Ircinal A, and Manzamines A and D," *J. Am. Chem. Soc.* **1998**, *120*, 6425-6426.

63. J. Winkler, Y. Kwak, "An Approach to Controlled Oligomerization via Iterative Diels-Alder Cycloadditions on Solid Supports," *J. Org. Chem.* **1998**, *63*, 8634-8635.

64. J. Axten, L. Krim, H. Kung, J. Winkler, "An Improved Synthesis of *dl-threo*-Methylphenidate. Preparation and Biological Evaluation of Novel Analogs," *J. Org. Chem* **1998**, *63*, 9628-9629.

65. J. Winkler, S. Kim, S. Harrison, N. Lewin, P. Blumberg, "Synthesis and Biological Evaluation of Highly Functionalized Analogs of Ingenol: The Importance of Hydrophobic Effects on Binding to Protein Kinase C," *J. Am. Chem. Soc.*, **1999**, *121*, 296-300.

66. G. Collins, L. Choi, K. Ewing, V. Michelet, C. Bowen, and J. Winkler, "Photoinduced Switching of Metal Complexation by Quinolinospiropyranindolines in Polar Solvents," *J. Chem. Soc., Chem. Commun.* **1999**, 321-322.

67. J. Winkler, J. Holland, J. Kasparec, and P. Axelsen, "Synthesis and Biological Evaluation of Constrained Epothilone Analogs: The Efficient Synthesis of Eleven-

Membered Rings by Olefin Metathesis," *Tetrahedron* (invited contribution to Symposium-in-Print on Olefin Metathesis in Synthesis) **1999**, *55*, 8199-8214.

68. J. Axten, R. Ivy, L. Krim, J. Winkler, "An Enantioselective Synthesis of d-threo-Methylphenidate, *J. Am. Chem. Soc.* **1999**, *121*, 6511-6512.

69. J. Winkler, E. Doherty, " The First Total Synthesis of (±)-Saudin, *J. Am. Chem. Soc.* **1999**, *121*, 7425-7426.

70. J. Winkler, E. Piatnitski, J. Mehlmann, J. Kasparec, P. Axelsen, "Design and Synthesis of Novel Foldamers Based on an Anthracene Diels-Alder Adduct," *Angew. Chem. Int. Ed. Engl.*, in press.